Universidade de Aveiro Departamento de Biologia
Ano 2012

Patrícia Aires Alegria

MUDANÇAS CLIMÁTICAS E A FLORAÇÃO DE ESPÉCIES SELECIONADAS DE PLANTAS

EFFECTS OF CLIMATE CHANGE ON FLOWERING TIMES OF SELECTED PLANT SPECIES

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada, ramo de Ecologia, Biodiversidade de Gestão de Ecossistemas, realizada sob a orientação científica do Prof. Doutor Paulo Cardoso da Silveira, Professor auxiliar do Departamento de Biologia e do Prof. Doutor Alfredo Moreira Caseiro Rocha, Professor associado com agregação do Departamento de Física da Universidade de Aveiro.

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palavras-chave

Alterações climáticas, floração, espécimes de herbário, fenologia, *Narcissus bulbocodium*, *Romulea bulbocodium*, *Erica australis*, *Erica arborea*, Portugal.

resumo

As temperaturas globais estão a aumentar rapidamente, promovendo mudanças importantes em comunidades de plantas e contribuindo para a perda global de biodiversidade. Das respostas biológicas para o aquecimento global, as mudanças no tempo dos eventos fenológicos como floração estão entre as mais sensíveis e importantes, tanto do ponto de vista biológico como económico. As fontes mais confiáveis de dados para monitorizar os eventos de ciclo de vida são os conjuntos de dados compilados sistematicamente por estações fenológicas. Embora estes sejam relativamente abundantes na Europa Central e do Norte, são escassos em Portugal. A falta de observações fenológicas pode, contudo, ser preenchida por coleções biológicas em herbários e museus, contanto que algum método de correção seja aplicado para superar as diferenças em pontos de amostragem. O objetivo principal deste trabalho foi encontrar um modelo que possa ser utilizado para corrigir as diferenças geográficas entre os locais de colheita em grandes áreas, permitindo a utilização de espécimes abundantes depositados nos herbários portugueses para o estudo dos efeitos do aumento da temperatura sobre a fenologia de plantas. Neste estudo, foram examinados espécimes de herbário de quatro espécies, distribuídos por todo o território Português. Duas bulbosas: *Narcissus bulbocodium* L. e *Romulea bulbocodium* Sebast (L.). & Mauri, Fl., e duas arbustivas: *Erica australis* L. e *Erica arborea* L. Uma relação não-significativa entre a data de floração e o ano foi observada para os modelos HBL e MLR, no entanto, os melhores ajustes foram obtidos ao utilizar o modelo MLR. Considerando a correlação entre JDN corrigido usando MLR e a temperatura, como se todos os espécimes fossem colhidos no CGP, apenas para *R. bulbucodium* foi obtida uma relação significativa (P = 0,019), observando-se um avanço de 9,6 dias por 1 °C. Esta correlação mostra o comportamento de espécies que enfrentam a mudança climática, o que permite a utilização desta espécie como um indicador para os efeitos das alterações climáticas na fenologia de plantas. Portanto, podemos usar modelo MLR para corrigir as diferenças geográficas entre os locais de colheita em grandes áreas. O uso de espécimes de herbário é recomendado como uma metodologia sólida, se houver cuidado em selecionar uma espécie com um tempo de floração curto e um vasto tamanho de amostra, podem vir a ser elucidados resultados significativos.

keywords

Climate change, flowering times, herbarium specimens, phenology, *Narcissus bulbocodium*, *Romulea bulbocodium*, *Erica australis*, *Erica arborea*, Portugal.

abstract

Global temperatures are increasing quickly, promoting important shifts on plant communities and contributing to the global loss of biodiversity. Of the biological responses to global warming, changes in the timing of phenological events such as flowering are among the most sensitive and important, both from a biological and economical point of view. The most reliable data sources to monitor life-cycle events are datasets systematically compiled by phenological stations. Although these are relatively abundant in Central and Northern Europe, they are scarce in Portugal. The lack of phenological observations can, however, be filled up by biological collections in herbariums and museums, as long as some correction procedure is applied to overcome differences in sampling locations. The main objective of this work was to find a model that can be used to correct for the geographical differences among the collection sites across large areas, allowing for the use of the abundant specimens held in the Portuguese herbaria for the study of the effect of the rise of temperature on plant phenology. In this study, we examined herbarium specimens of four species, distributed by the whole of the Portuguese territory. Two bulbous: *Narcissus bulbocodium* L. and *Romulea bulbocodium* (L.) Sebast. & Mauri, Fl., and two shrubs: *Erica australis* L. and *Erica arborea* L. A non-significant relationship between flowering date, adjusted using Hopkins Bioclimatic Law or Multiple Linear Regression (MLR), and year was observed, however, best fits were obtained using MLR. On the other hand, the correlation between JDN (corrected using MLR) and temperature (adjusted as if all specimens were collected in GCP), was found to be significant (P = 0,019) for *R. bulbucodium*, indicating an advance in flowering date of 9,6 days per 1 °C for this species. This correlation shows the behavior of species facing climate change, which allows the use of this species as an indicator for climate change effects on plant phenology. Therefore, we can use MLR Model to correct for the geographical differences between collection sites across large areas. The use of herbarium specimens is recommended as a robust methodology, if care is taken to select a species with a short flowering time and a large sample size from which significant results can be elucidated.

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ABBREVIATIONS

ALGU - Herbarium of University of Algarve

AVE - Herbarium of University of Aveiro

COI - Herbarium of University of Coimbra

ELVE - Herbarium of National Station for Plant Breeding

GCP - Geodetic Center of Portugal

HBL - Hopkins's Bioclimatic Law

IPG - International Phenological Garden

JDN - Julian Day Number

LISE - Herbarium of National Agronomic Station

LISI - Herbarium of Institute of Agronomy of the Technical University of Lisbon

LISU - Herbarium of University of Lisbon

MLR - Multiple Linear Regression

PO - Herbarium of University of Oporto

CHAPTER 1.

Introduction

Objectives of the thesis

Numerous studies show that the temperature increase affects biological processes in plants (Cleland *et al.*, 2007)

Given the scarcity of information on the effects of climate change on plant phenology of the Flora of Portugal we intend to conduct a study that brings together a solid knowledge on this subject.

At first instance, we aimed to study the effects of climate change on flowering times of *Narcissus bulbocodium* L., which is an abundant species in Portugal and has a short flowering period. We used specimens deposited in Portuguese herbaria to test a model based on Hopkins's Bioclimatic Law (HBL) (Hopkins, 1938) that can be used to correct the geographical differences between the collection sites.

In a second step, we proposed to expand this study to other species and tested another model, based on Multiple Linear Regression (MLR), to answer the following question: how can we adjust collecting data in order to infer the effect of climate change on species flowering times across large areas? Four species, representing two distinct life forms, were selected to test the model. Two bulbous: *Narcissus bulbocodium* L. and *Romulea bulbocodium* (L.) Sebast. & Mauri, Fl., and two shrubs: *Erica australis* L. and *Erica arborea* L. The first two species are well adapted to different climates and environments, are common in the Portuguese territory and its flowering periods are short and early in the year. The last two, although having flowering periods not as short as that of the first ones, they are equally well adapted and very frequent across the study area. Therefore, allowing its use to draw conclusions about the use of tested procedures of adjustment of flowering dates across large areas.

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CHAPTER 2.

Effects of climate change on flowering times of *Narcissus bulbocodium* L. in Portugal

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Abstract

Global temperatures are increasing at an unprecedented rate, promoting important shifts on plant communities and contributing to the global loss of biodiversity. Of the biological responses to global warming, changes in the timing of phenological events such as flowering are among the most sensitive and important, both from a biological and economical point of view. The most reliable data sources to monitor life-cycle events are datasets systematically compiled by phenological stations. Although these are relatively abundant in Central and Northern Europe, they are scarce in Portugal. The lack of phenological observations can, however, be filled up by biological collections in herbariums and museums. These specimens are potential sources of long-term data to detect changes in flowering phenology, as long as some correction procedure is applied to overcome differences in sampling locations. In this study, we examined herbarium specimens of *Narcissus bulbocodium* L., an early flowering species with a short flowering period, collected between 1882 and 2006 and, more or less, equally distributed by the whole of the Portuguese territory. The main objective of this work is to find a model that can be used to correct for the geographical differences among the collection sites, allowing for the use of the abundant specimens held in the Portuguese herbaria for the study of the effect of the rise of temperature on plant phenology.

Keywords climate change; flowering times; herbarium specimens; *Narcissus bulbocodium*; phenology; Portugal

Introduction

Global warming is already affecting natural processes around the world. Examples of observed changes include melting of glaciers, thawing of permafrost, earlier break-up of ice on rivers and lakes, species are moving their ranges towards the poles and up mountain slopes, lengthening of growing seasons, earlier flowering of plants, emergence of insects and egg-laying in birds and some species are, even, going extinct (Walther *et al.*, 2002, Parmesan & Yohe, 2003). Some of the above mentioned effects, namely periodic events in the life cycles of animals or plants, as influenced by the environment, especially seasonal variations in temperature and precipitation, are studied by Phenology (Cleland *et al.*, 2007).

The phenological changes from year to year may be a sensitive and easily observable indicator of environmental changes, and have a wide range of consequences for ecological processes, agriculture, forestry, human health, and the global economy. The wealth of historical phenological records allows scientists to examine trends from the past and make cautious predictions about what may happen to species in the future (Khanduri Sharma & Singh, 2008). The seasonal onset of warmer temperatures triggers a suite of physiological responses in plants species, such as leaf bud-burst and the initiation of flowering. These species are prime candidates on which to base monitoring programs to elucidate the effect of climate change on natural systems (Gallagher Hughes & Leishman, 2009). In recent years, phenology studies have been conducted in many plant taxa from a broad range of biogeographic regions, including Asia (Aono & Kazui, 2008), Australia (Gallagher *et al.*, 2009, Keatley *et al.*, 2002), North America (Lavoie & Lachance, 2006, Miller-Rushing *et al.*, 2006, Primack *et al.*, 2004) and Europe (Ahas *et al.*, 2002, Menzel, 2000, Menzel *et al.*, 2006, Ziello *et al.*, 2009, Clark & Thompson, 2010).

The first phenological network in Europe is linked with the name of Carl von Linné, who made his observations in Sweden (Menzel, 2003). The International Phenological Garden (IPG) is nowadays a unique system in Europe, which was founded by F. Schnelle and E. Volkert in 1957 (Menzel, 2003). The current network ranges across 28° of latitude from Scandinavia to Macedonia and across 37° of longitude, from Ireland to Finland in the North and from Portugal to Macedonia in the South. It consists of 89 stations in 19 European countries (updated in 2010). Since 2000, the observation programme includes 8 phenophases of 21 plant species (Chmielewski, 2010).

Not included in the IPG network, in 1942, the Spanish Instituto Nacional de Meteorología (INM) created its own phenological network in Spain to gather information about plant and animal phenology. This network relies on volunteer observers that record several phenological events according to standardized observational methods and a selected species list. Consequently, the monitoring of phenological events is widespread throughout Spain (Gordo & Sanz, 2009).

On the contrary, phenological observations in Portugal are rather scarce. The study of phenology in Portugal began in 1759, by Domingos Vandelli under request by Carl von Linné (Bettencourt, 1982), however nobody knows the results. From 1876 to 1903 regular observations were conducted by Filipe de Figueiredo from the Institute of Agriculture that resulted in a manual of phenology (Bettencourt, 1982). In 1956, phenological observations where started at the Instituto Geofísico do Porto. In 1968, this station was the first Portuguese phenological observatory to integrate the IPG network. However, at present, this observatory is deactivated (Chmielewski, 2010, Bettencourt, 1982).

At present, mainland Portugal has only one active phenological station, in Évora, which was incorporated into the IPG network in 2004 (Chmielewski, 2010).

Therefore, phenological studies in Portugal are practically fragmented. However, other kinds of data sources can be used. Data collected from historical records have been used expansively to investigate the impacts of climate change on plant species such as herbarium specimens (Gallagher *et al*., 2009, Primack *et al*., 2004, Miller-Rushing *et al.*, 2006, Lavoie & Lachance, 2006, Robbirt *et al.*, 2011) and photographic archives (Aono & Kazui, 2008, Crimmins & Crimmins, 2008). Herbarium plant specimens, i.e. plants, or plant parts, dried, fixed in cardboards, properly labeled, cataloged and arranged according to botanical classification (Pinho *et al.*, 2003) are unique amongst these sources of information in that they capture an individual plant's phenological state at the time and location of collection, and therefore may represent a substitute for field observation (Robbirt *et al*., 2011). If used carefully, i.e. using some kind of correction for the different climatic conditions associated with sampling locations, this information can be used in order to detect changes in the timing of life-cycle events. Lavoie and Lachance (2006) used the date of disappearance of snow cover to make the above mentioned correction, however this cannot be applied to mainland Portugal.

Therefore, the main objective of this work is to find a model that can be used to correct for the geographical differences among the collection sites, allowing for the use of the abundant specimens held in the Portuguese herbaria for the study of the effect of the rise of temperature on plant phenology.

Our attempt uses the Hopkins's Bioclimatic Law, which states that there is a relationship between phenological events of plants and animals with the various elements which make up the climate of the region such as elevation, latitude and longitude and that spring advances 4 days for each 1° latitude, 5° longitude and 400 feet of altitude (Hopkins, 1938).

We selected as a model species *N. bulbocodium*, because this species is well adapted to different climates and environments, is very frequent and widespread in the Portuguese territory and its flowering period is short and early in the year. Furthermore, since it is a geophyte, there is a higher probability that its phenological behavior might reflect climatic changes. Finally, in recent decades, it has been abundantly collected and deposited in Portuguese herbaria, permitting availability of data for this study.

Materials and Methods

Study area

Mainland Portugal is located in the extreme southwest Europe, at the Iberian Peninsula, roughly between 37° to 42°N and 6,5° to 9,5°W, a territory that extends about 580 km N to S and 220 km E to W (Fig. 2.1).The region is characterized by altitudes ranging from 0 to 2000 m. North and Central Portugal have significant areas that exceed 1000 m (Miranda *et al.*, 2006). This territory is part of the Mediterranean biogeographical region except the North Coast, inserted in the Atlantic region (European Environment Agency, 2002).

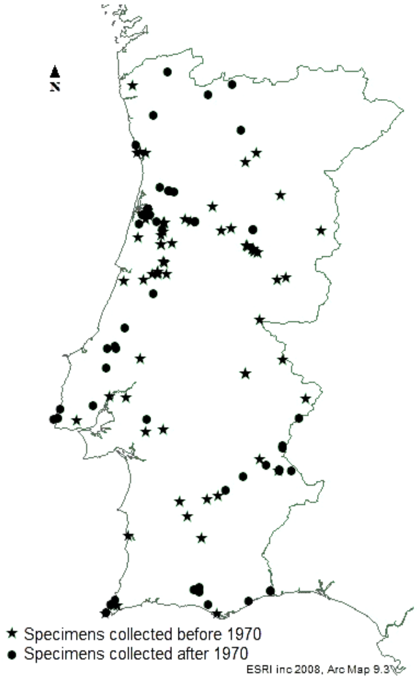

Fig. 2.1 Geographic distribution of used herbarium specimens of *N. bulbocodium* in the study area.

Climate data

According to the international classification by Köppen, the climate of mainland Portugal is divided in two regions: a temperate climate with wet winters and dry and hot summers (mostly North West) and another temperate climate with wet winters and dry but moderately hot summer (mostly South East) (Instituto Meteorologia I.P, 2008). Observed precipitation in the North West region is relatively high, reaching, in some places, a mean annual accumulated rainfall exceeding 3000 mm. In several areas of the South East, by the contrary, the accumulated annual precipitation does not exceed an average of 500 mm. The average annual temperature varies between 7 °C in the highlands of central and northern regions and 18 °C in the south coast (Miranda *et al.*, 2006, Instituto Meteorologia I.P, 2008).

Species data

Belonging to the family Amaryllidaceae, *Narcissus bulbocodium* L. (petticoat daffodil) is an endemic species of the South and West of France, Iberian Peninsula, and North Africa. It is a species that occurs throughout the Portuguese mainland. It is an herbaceous plant, bulbous, with 8-35 cm. It has large flowers, solitary, of a light yellow color. Occurs in meadows, high altitude grasslands, sand-hills, and clearings of heath, fields of rock-roses and deciduous and perennial woods dominated by *Quercus* species. The flowering period is short and early in the year, beginning in February and ending in May or June (Aedo, 2011). Currently, this species is protected by Habitats Directive 92/43/EEC, Annex V, considered a species of community interest (EU, 1992).

Herbarium data

After consulting the main Portuguese herbaria, we selected a total of 122 specimens of *N. bulbocodium*. From the University of Coimbra (COI) we selected 34 specimens, 27 from the University of Aveiro (AVE), 15 from the National Museum of Natural History, University of Lisbon (LISU), 14 from the Institute of Agronomy of the Technical University of Lisbon (LISI), 13 from the National Agronomic Station (LISE), 9 from the University of Oporto (PO), 5 from the University of Algarve (ALGU) and 5 from the National Station for Plant Breeding (ELVE). All specimens collected on the Portuguese mainland, at peak flowering, with complete date of harvest where selected. We rejected the hybrids and specimens with only fruit, or flower and fruit. In order to use only specimens with exact information concerning the latitude, longitude and altitude of the place of collection we established a criterion based on Hopkins's Bioclimatic Law, admitting, at most, an error of 1 day. That is, all specimens whose geographic coordinates and altitude could be determined with an error smaller than 27,83 km of latitude, 106,25 km longitude and 30,48 m in altitude, were included in the selection, excluding those who exceed this error. Taking into account the criterion mentioned above, we determined the altitude and geographical coordinates (degrees, minutes, seconds) using the software Google Earth 2010 (version 5.2.1.1588). Using the software ArcMap 9.3 (ESRI) the geographic distribution of the studied specimens was analyzed, to check if they were well distributed by the Portuguese territory and between two time periods: 1882-1970 and 1971-2006.

Meteorological data

Due to the difficulty in obtaining weather records for the period from 1882 to 2006, along the Portuguese territory, we used only records from the meteorological station of the Geophysical Institute of Lisbon (ECA, 2011). For this study, according to the peak flowering of *N. bulbocodium*, we calculated the average of the first four months of each year (January, February, March and April), corresponding to the year of collection. Due to the lack of meteorological data, specimens collected in 1982 where not used. Considering that the temperature decreases an average of 6,5°C per 1000 m of altitude a proportional correction was introduced to determine an estimated mean temperature for each collecting point based on its altitude.

Analysis

For each specimen, a Julian Day Number (JDN) was computed based on its collecting date. Considering that the specimens used where collected at different locations, a correction was introduced, based on Hopkins's Bioclimatic Law (1938) i.e., to the calculated JDN, 4 days where added/subtracted for each 1° latitude, 5° longitude and 400 feet (121,92 m) altitude. The JDN of all *N. bulbocodium* collecting sites was this way converted as if they have all been collected in the Geodetic Center of Portugal (39°41'40,20619''N; 8°7'50,06228''W; 580 m altitude). Data analysis in the study, statistical calculations and the correlations and their regressions were performed using the software Microsoft Excel 2010 (version 14.05128.5000).

Results

The climate in Lisbon has become progressively warmer since the XIXth century. The mean annual temperature recorded at the Meteorological station of the Geophysical Institute of Lisbon, from 1864 to 2008 (Fig. 2.1), rose by approximately 1,8 degrees Celsius in 144 years (y = 0,0125x -7,9105; R^2 = 0,5713; P <0,0001). Also there was an increase of 1,8 °C in the mean January to April temperature (Fig. 2.2) for the same interval of time (y = 0,0128x -12,211; R^2 = 0,3973; P <0,0001).

A great climatic variability was observed, however, along the years. In the mean annual temperature, three high peaks reaching 17,7 °C, where observed, in 1955, 1995 and 1997. The lower peak (14,6 °C) was observed in 1889. In the January to April mean temperatures a high peak was observed in 1997, reaching 15,4 °C, and two lower peaks where observed in 1888 and 1934 with a temperature of 10,5 °C.

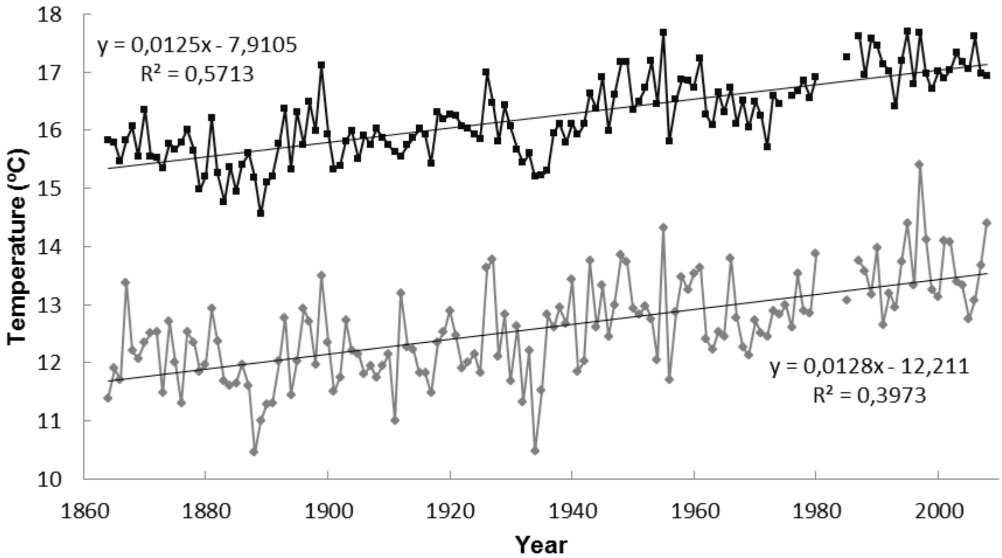

Fig. 2.2 Lisbon temperatures from 1864 to 2008 as reported by Meteorological station of the Geophysical Institute of Lisbon. The top series represents mean annual temperatures. The bottom series represents mean temperatures in January, February, March and April. The lines are the best fit lines for the series.

After correction using Hopkins's Bioclimatic Law, an advancement of 1,5 days per decade in flowering time of *N. bulbocodium* was observed (Fig. 2.3). This has resulted in an total advancement of 18,8 days since 1882. However, the relationship between flowering date and year was not significant (y = -0,1483x + 364,61; R^2 = 0,0106; P = 0,260037; n= 122).

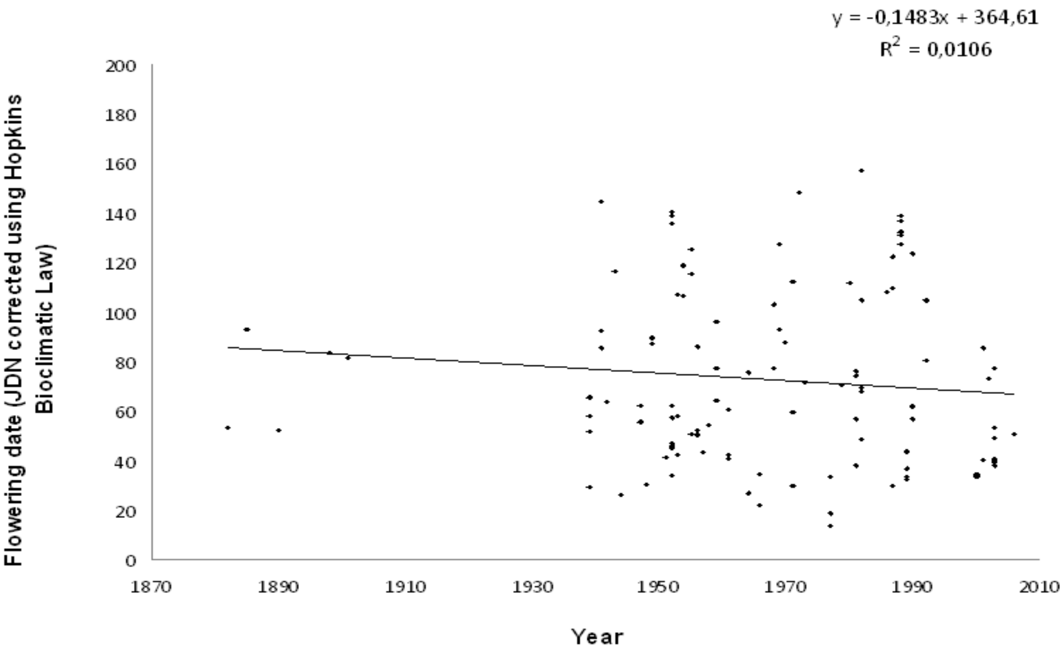

Fig. 2.3 Relationship between the day number that a flowering herbarium specimen of *N. bulbocodium* was collected (corrected using Hopkins Bioclimatic Law) and year.

On the contrary, the relationship between flowering date of herbarium specimens (JDN) corrected by Hopkins's Bioclimatic Law and the average temperature in January-April (Fig. 2.4), in the period from 1882 to 2006, was significant (y = -8,5904x + 170,73; R^2 = 0,4401; P <0,0001; n= 122), reaching an advancement in flowering time of 8,6 days per 1 °C.

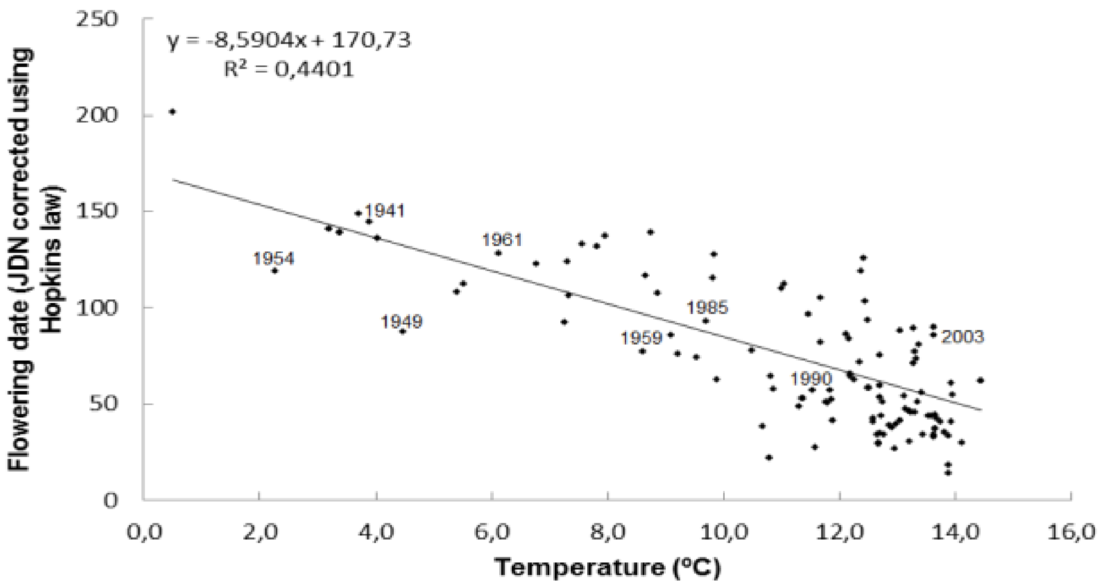

Fig. 2.4 Relationship between the day number that a flowering herbarium specimen of *N. bulbocodium* was collected (corrected using Hopkins Bioclimatic Law) and mean temperature of the first four months of the year, Jan-Apr. The line is the best fit line for the series.

Discussion

The present study showed that mean annual temperatures and mean January to April temperatures has risen, in Lisbon, by 1,8 °C in 144 years, i.e. 0,125 °C per decade. This corroborates well with what has been reported by IPCC (2007) for the global rise of temperature (0,13 °C per decade from 1956 to 2005) and with the reported mean temperature increase for Portugal, in the period 1941 to 2005, of 0,11 ºC per decade (Marques & Antunes, 2009).

Has expected, this rise in temperature caused an advancement in the flowering dates of *N. bulbocodium* across the Portuguese territory, estimated in 1,5 days per decade, resulting in a total advancement of 18,8 days since 1882.

Although the relationship between flowering date and year was not significant, it is within the range reported in the study by Khanduri *et al*. (2008). This author, reviewing the published literature, reported and average advancement of 1,9 days per decade, ranging from 0,2 to 4,5 days per decade, depending on the author, species and geographical and time scope of the study under analysis (Khanduri *et al*., 2008).

The dispersion of the records was, however, high (Fig. 2.3), probably due to the climatic variability between different years and collection sites, resulting in a non-significant relationship between flowering date and year. The relationship between flowering date of herbarium specimens (JDN) corrected by Hopkins's Bioclimatic Law and the average temperature in January-April (Fig. 2.4), was, however, highly significant. This suggest that the relationship between flowering dates and year was not significant mostly due to climatic variability between years, and not due to differences between collection sites, therefore indicating that we can use Hopkins's Bioclimatic Law to correct for the geographical differences between collection sites across large areas.

The fact that the used herbarium specimens where acceptably distributed in a North-South and East-West gradient in the study area before and after 1970 (Fig 2.1.) is important to demonstrate that there where no biases introduced in the analysis due to asymmetric sampling.

A small amount of specimens collected before 1939 was used in the analysis due to the general lack of an indication of day of collection in the specimens from the XIXth century. Also, specimens from the period 1900 to 1938 are practically inexistent in the Portuguese herbaria. However, the abundant specimens collected after 1939 (inclusive) allowed a robust analysis, especially of the relationship between flowering dates and mean January to April temperatures. In fact, we found that flowering date of *N. bulbocodium* advanced 8,6 days per 1°C in the period from 1882 to 2006. This is within the range of values reported in other studies. For example, Robbirt *et al*. (2011) reported an advancement of 5,7 to 6,7 days per 1 °C rise in temperature, for *Ophris sphegodes*; Gallagher *et al*. (2009) reported an advancement varying from 4,35 to 11,97 days per 1 ºC on Australian alpine species and Primack *et al*. (2004) reported a mean advancement of 3,9 days per 1 °C in a study including 229 species in Boston.

In this study we considered corrections of altitude, longitude and latitude, based on Hopkins Bioclimatic Law, but other variables such as rain and humidity may also influence flowering times. Furthermore, errors related to the spatial accuracy of coordinates resulting from misrepresentation of the original location of the collection or lack of details regarding slope, aspect and soil type in the label of the collection might have contributed to the high dispersion of the records and non-significance of the regression concerning the relationship between flowering times and year.

N. bulbocodium, as a spring-flowering plant, with a short and early flowering period (Aedo, 2011), falls into a group identified as having flowering phenologies that are likely to be particularly sensitive to temperatures early in the year (Robbirt *et al.*, 2011). This fact is important for the phenological study and is supported by the results of this study.

The earlier onset of flowering can have consequences not only for the individual plants and populations affected, but also to the maintenance of diversity at the community level. Understanding the response of primary producers within communities provides scope for investigating the effect of climate change on biotic interactions with species at other trophic levels (Gallagher *et al.*, 2009). For most species of plants and animals, biological collections are the only source of long-term phenological data. It is estimated that some 2,5 billion specimens of flora and fauna are held in biological collections worldwide (Graham *et al.*, 2004). With appropriate validation, the exploitation of this resource will have increasing relevance and value as we seek to understand and predict the consequences of continuing climate change (Robbirt *et al.*, 2011).

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CHAPTER 3.

Testing two methods to adjust herbarium collecting data in order to infer the effect of climate change on species flowering times across large areas

Abstract

Climate change has been documented through numerous studies and climate is expected to continue changing in coming time. The flowering times of herbarium specimens can be used to measure biological responses to changes in climate over time. The main objective of this work is to test two methods to adjust herbarium collecting data in order to infer the effect of climate change on species flowering times across large areas. In this study, we examined herbarium specimens of four species collected between 1882 and 2006. Two bulbous: *Narcissus bulbocodium* L. and *Romulea bulbocodium* (L.) Sebast. & Mauri, Fl., and two shrubs: *Erica australis* L. and *Erica arborea* L. The first two species are well adapted to different climates and environments, are common in the Portuguese territory and its flowering periods are short and early in the year. The last two, although having flowering periods not as short as that of the first ones, they are equally well adapted and very frequent across the study area. After adjusting flowering times (JDN) for variables: altitude, latitude and longitude with the HBL and MLR Models, best fits were obtained when using the MLR model, however we have not obtained statistically significant correlations between JDN and year. To check possible relationships between flowering dates and changes in temperature we analyzed the records of 5 weather stations and, besides evaluating the evolutionary trend since the nineteen century, we verified possible relationships between this variable and flowering dates. In Portugal the annual average temperatures increased by 1,8 °C in 126 years. By correlating the original flowering dates and mean temperature, corrected to the altitude of collecting site (-6,5 °C / 1000 m), a significant relationship was observed, indicating that flowering of the bulbous species advances 5 days per 1 °C increase in the mean temperature of the 4 months before flowering at each collecting site, and advances 11 days per 1 °C increase in the shrubs. To further investigate the behavior of species facing climate change we analyzed the relationship between flowering dates, adjusted using MLR, and temperature, also adjusted as if each specimen had been observed in the GCP, and a significant ($P = 0,019$) relationship was obtained for *R. bulbocodium*, indicating an advance of 9,6 days per 1 ºC increase, and, thus, allowing the use of this species as an indicator for climate change effects on plant phenology. The use of herbarium specimens is recommended as a robust methodology if care is taken to select a species with a short flowering time and a large sample size from which significant results can be obtained.

Keywords climate change; flowering times; herbarium specimens; phenology; *Narcissus bulbocodium; Romulea bulbocodium; Erica australis; Erica arborea*

Introduction

Climate change has been documented through numerous studies (Intergovernmental Panel on Climate Change, 2007) and effectively understanding the effects of recent climate change is an essential step to predict the consequences of future changes in species, species interactions and ecosystems consecutively (Robbirt *et al.*, 2011).

Changes in the timing of phenological events are among the most important indicators of global warming (Parmesan & Yohe, 2003). The flowering times of several species appear sensitive enough to changes in temperature that they could serve as indicator species and be used to measure biological responses to changes in climate over time (Miller-Rushing & Primack, 2008).

Several more recent studies have demonstrated that herbarium specimens alone can provide a statistically robust dataset for analyses in climate change (Primack *et al.*, 2004, Miller-Rushing *et al.*, 2006, Gallagher Hughes & Leishman, 2009, Robbirt *et al.*, 2011, Panchen *et al.*, 2012, Diskin *et al.*, 2012). Although preliminary methodologies have been developed (Primack *et al.*, 2004), a careful examination of the databases of herbarium specimens should be undertaken prior to analysis to detect biases or trends associated with sampling locations (Lavoie & Lachance, 2006). Changes in biological and physical systems essentially occur in regions of detected temperature increase, which itself cannot be explained by natural climate variations alone (Rosenzweig *et al.*, 2008). Ziello *et al.* (2009) find that the external dependence on altitude induces a direct connection between temporal trends in temperature and in phenology.

Specimens from a large geographic area can be analyzed however may require the use of correction procedures (Diskin *et al*., 2012). Lavoie and Lachance (2006) used herbarium specimens and estimated early flowering over time. Gallagher *et al*. (2009) evaluated early flowering responses with respect to increasing temperature. Robbirt *et al.* (2011) studied the flowering time responses by spring temperature using herbarium and field observations, separately. All these herbarium-based phenological studies utilized flowering time as response variable and used linear regression model as statistical application. However, the Generalized Additive Model (GAM) was selected as an appropriate approach to address these issues by Gaira *et al*. (2011).

Therefore, we proposed to answer the following question: how to correct collecting data in order to infer the effect of climate change on species flowering times across large areas? In this study we first tried the Hopkins's Bioclimatic Law (HBL) (Hopkins, 1938) as a possible model, to correct herbarium specimens data. Because the results showed some improvement, but were not completely satisfactory we then tried the Multiple Linear Regression (MLR), as a possible new model, to correct herbarium specimens data.

Four species, representing two distinct life forms, were selected to test the model. Two bulbous: *Narcissus bulbocodium* L. and *Romulea bulbocodium* (L.) Sebast. & Mauri, Fl., and two shrubs: *Erica australis* L. and *Erica arborea* L. The first two species are well adapted to different climates and environments, are common in the Portuguese territory and its flowering periods are short and early in the year. The last two, although having flowering periods not as short as that of the first ones, they are equally well adapted and very frequent across the study area.

Material and Methods

Study area

Mainland Portugal is located in the extreme southwest Europe, at the Iberian Peninsula, roughly between 37° to 42°N and 6,5° to 9,5°W, a territory that extends about 580 km N to S and 220 km E to W (Fig. 3.1). The region is characterized by altitudes ranging from 0 to 2000 m. North and Central Portugal have significant areas that exceed 1000 m (Miranda *et al.*, 2006). The north of the river Tejo appears very uneven, with the exception of coastal plains, with an average altitude above 400 m, intersected by valleys and rivers with significant flow rates. Meanwhile, south of the Tejo relief is gently rolling hills, with altitudes weak, dominated the plains. The mountains with higher altitude are situated north of the Tejo. The relief Portuguese is complete with a fringe of coastal plains (Infopédia, 2012). This territory is part of the Mediterranean biogeographical region except the North Coast, inserted in the Atlantic region (European Environment Agency, 2002).

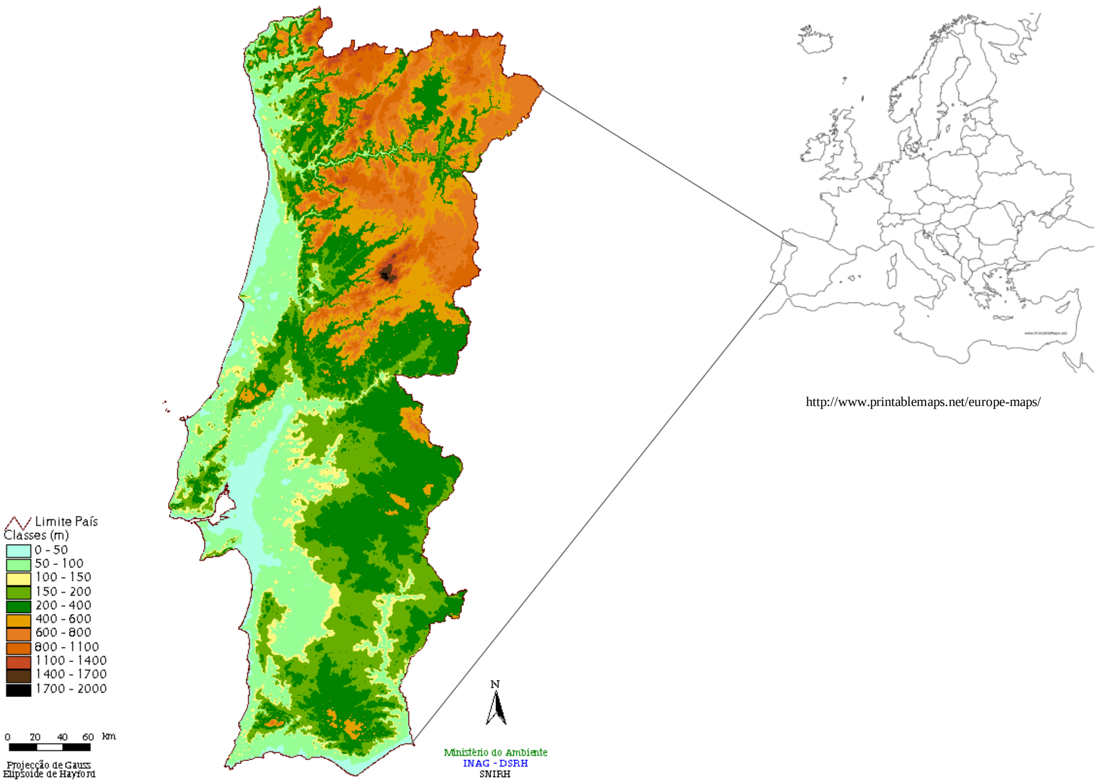

Fig. 3.1 Context of the Portuguese territory in Europe evidencing the hypsometric letter of mainland Portugal.

Climate data

The climate of mainland Portugal is divided in two regions: a temperate climate with a wet winter and dry and hot summer (mostly North West) and another temperate climate with a wet winter and dry but moderately hot summer (mostly South East), according to the international classification by Köppen (Instituto Meteorologia I.P, 2008). Observed precipitation in the North West region is relatively high, reaching, in some places, a mean annual accumulated rainfall exceeding 3000 mm. In several areas of the South East, by the contrary, the accumulated annual precipitation does not exceed an average of 500 mm. The average annual temperature varies between 7 °C in the highlands of central and northern regions and 18 °C in the south coast (Miranda *et al.*, 2006, Instituto Meteorologia I.P, 2008).

Study Species

Narcissus bulbocodium L.

Belonging to the family Amaryllidaceae, petticoat daffodil (Fig. 3.2 a) is an endemic species of the South and West of France, Iberian Peninsula, and North Africa. It is a species that occurs throughout the Portuguese mainland. It is an herbaceous plant, bulbous, with 8-35 cm high. It has large, solitary flowers, of a light yellow color. Occurs in meadows, high altitude grasslands, sand-hills, and clearings of heath, fields of rock-roses and deciduous and perennial woods dominated by *Quercus* species. The flowering period is short and early in the year, beginning in February and ending in May or June (Aedo, 2011). Currently, this species is protected by Habitats Directive 92/43/EEC, Annex V, considered a species of community interest (EU, 1992).

Romulea bulbocodium (L.) Sebast. & Mauri, Fl.

Member of the family Iridaceae, the violet romulea (Fig. 3.2 b) is native to the Mediterranean region, throughout the Iberian Peninsula, mainly in the western half. It is a species that occurs throughout the Portuguese mainland, except Alto Alentejo, Ribatejo and Trás-os-Montes. It is a herbaceous plant, bulbous, with 17-30 cm high. It has large flowers, solitary, of a violet color with a white-yellow tube. Occurs in grasslands, clearings of the holm-oak forests, pine forests, *Quercus pyrenaica* forests, heathlands, often in acid or decarbonated substrates, grazed or altered. The flowering period is short and early in the year, beginning in February and ending in April (Castroviejo *et al.*, 1993b).

Erica australis L.

Spanish heath (Fig. 3.2 c), of the Ericaceae, is an endemic species of the Iberian Peninsula, and Northwest Africa. It is a species that occurs throughout the Portuguese mainland. It is an evergreen shrub, reaching 2,5 m. The inflorescences develop at the apex of lateral branches and are umbelliform, with 2-6 flowers, the corollas are pink, with 10 mm, of tubular to bell-shaped. Occurs in heathlands, shrublands and open forests, in siliceous or sometimes ultrabasic substrates. The flowering period begins in January and ends in May (Castroviejo *et al.*, 1997).

Erica arborea L.

Belonging to the family Ericaceae, tree heath (Fig. 3.2 d) is native to the Mediterranean region, Macaronesia, North and East Africa. It is a species that occurs in many Portuguese regions, including the Algarve, Beira Alta, Baixo Alentejo, Beira Litoral and Estremadura. It is a shrub or small evergreen tree. It develops numerous inflorescences at the apex of lateral branches, umbelliform in shape, with 1-3 white small bell-shaped flowers. Occurs in open forests, cool and dark shrublands, preferably in siliceous soils. The flowering period begins in February and ends in July (Castroviejo *et al.*, 1997).

Fig. 3.2 a – Flower of *N. bulbocodium;* **b** – Flower of *R. bulbocodium*; **c** – Inflorescences of *E. australis*; **d** – Inflorescences of *E. arborea*.

Herbarium data

After consulting the main Portuguese herbaria, we selected a total of 117 specimens of *N. bulbocodium,* 70 specimens of *R. bulbocodium,* 70 specimens of *E. australis* and 51 specimens of *E. arborea* as seen in Table 3.1. For all selected species used the methodology described in Herbarium data in Chapter 2.

Table 3.1 Number of specimens of *N. bulbocodium*, *R. bulbocodium*, *E. australis* and *E. arborea* selected from the herbaria: University of Algarve (ALGU), University of Aveiro (AVE), University of Coimbra (COI), National Station for Plant Breeding (ELVE), National Agronomic Station (LISE), Institute of Agronomy of the Technical University of Lisbon (LISI), University of Lisbon (LISU) and University of Oporto (PO).

Herbaria	***Narcissus bulbocodium***	***Romulea bulbocodium***	***Erica australis***	***Erica arborea***
ALGU	5	-	-	-
AVE	27	4	6	2
COI	30	6	17	8
ELVE	5	-	-	-
LISE	12	19	16	10
LISI	14	10	21	21
LISU	15	12	3	2
PO	9	19	7	8
n	117	70	70	51

Adjustment using Hopkins's Bioclimatic Law

For each specimen a Julian Day Number (JDN) was computed based on its collecting date. Considering that the specimens used where collected at different locations, a correction was introduced, based on Hopkins's Bioclimatic Law (Hopkins, 1938) defined in Analysis, Chapter 2. The JDN of all specimen´s collecting sites was then adjusted as if they had been collected in the Geodetic Center of Portugal (GCP, 39°41'40,20619''N; 8°7'50,06228''W; 580 m altitude).

Adjustment using the Multiple Linear Regression

A multiple linear regression (MINITAB® Release 14.20) was performed using original JDN as response value and Latitude, Longitude, Altitude and respective logarithms as predictive terms. From the results of the *Stepwise Regression,* a *Regression Analysis* was carried out obtaining a different equation for each species. Using the regression equation obtained for each species the JDN was recalculated resulting in a JDN expected by this model. The JDN of all specimen´s collecting sites was thus converted as if they have all been collected in the Geodetic Center of Portugal (GCP, 39°41'40,20619''N; 8°7'50,06228''W; 580 m altitude). This conversion was achieved using the following equation: $JDN_{Cor.gcp} = JDN_{Orig} - (JDN_{Exp}\text{-}JDN_{Exp.gcp})$. Where $JDN_{Cor.gcp}$ is the final value of JDN after correction, JDN_{Orig} is the original JDN as calculated from the herbarium specimen collecting date, JDN_{Exp} is the expected JDN calculated using the MLR equation for each collecting site's altitude and geographic coordinates and $JDN_{Exp.gcp}$ is the expected JDN calculated, using the same equation, for the GCP's altitude, latitude and longitude.

Meteorological data

The recorded herbarium specimens of the four species used for this study covered a period from 1882 to 2006. To cover the entire Portuguese territory in that period we used the records of mean monthly temperatures of the following weather stations: Geophysical Institute of Lisbon, Meteorological Observatory of the Serra do Pilar (Geophysical Institute), Meteorological Observatory of the Penhas Douradas, Geophysical Institute of the University of Coimbra and Faro Weather Station / Airport (ECA, 2011, NASA, 2011, NOAA, 2011).

Considering that the temperature decreases an average of 6,5 °C per 1000 m of altitude, a proportional correction was introduced to determine an estimated mean temperature for each collecting point based on its altitude (Petersen Sack & Gabler, 2011). The data for each station were corrected as if they were all registered to the altitude of CGP. For each month, a mean was estimated for the entire Portuguese territory, based on the records of the mean of the same month for all weather stations. For each species, the mean temperature of the month that includes the peak flowering plus the three previous months was used. For *N. bulbocodium* and *R. bulbocodium* the December-March mean was used, for *E. australis* January-April and for *E. arborea* February-May.

Other data analysis in the study, statistical calculations and the correlations and their regressions were performed using the software Microsoft Excel 2010 (version 14.0.6123.5001) and IBM SPSS (version 20) for Windows.

Results

In this study, the flowering period for the four species ranges from January to August. *N. bulbocodium* and *R. bulbocodium* bloomed between February and May, there was a flowering peak for both species in March. *E. australis* presented its flowering period between January to August with a peak in April. Finally, *E. arborea* bloomed between February and August and its flowering peak was in May.

Most of the relationships analyzed in this study were non-significant (Table 3.2). However, they show plausible trends, mostly in agreement with results reported by other authors, and they enabled an evaluation of the methods applied.

Table 3.2 Least-squares linear regression models of flowering observation with year of collection. Number of observation (*n*), *F*-test statistic, R^2 goodness of fit *P*-value and disturbance term (α). Direction of the trend is indicated by β value where negative numbers represent earlier flowering, and positive numbers represent later flowering. Standard error of β is also provided as a measure of confidence in estimates of slope. Type of analysis: JDN corrected using Hopkins Bioclimatic Law (Adj. H.B. Law) and JDN corrected using the Multiple Linear Regression (Adj. MLR).

Type of analysis Species	*n*	R^2	*F*	*P*	β	s.e. of β	α
Original data							
N. bulbocodium	117	0,0216	2,5332	0,1142	-0,1545	0,0876	383,72
R. bulbocodium	70	0,0005	0,0337	0,8548	-0,0262	0,1031	129,90
E. australis	70	0,0219	1,5226	0,2215	0,4191	0,0424	-714,81
E. arborea	51	0,0842	4,5069	0,0388	0,0262	0,0581	-1205,60
Adj. H.B. Law							
N. bulbocodium	117	0,0089	1,0380	0,3104	-0,082	0,1092	249,11
R. bulbocodium	70	0,0021	0,1424	0,7071	-0,0473	0,1172	181,54
E. australis	70	0,0046	0,3132	0,5775	0,1458	0,0562	-180,84
E. arborea	51	0,0560	2,9087	0,0944	0,4207	0,0781	-689,33
Adj. MLR							
N. bulbocodium	117	0,0263	3,1067	0,0806	-0,1396	0,1069	362,86
R. bulbocodium	70	0,0063	0,4315	0,5135	-0,0835	0,1150	252,04
E. australis	70	0,0008	0,0569	0,8121	-0,0546	0,0642	208,05
E. arborea	51	0,0200	0,9988	0,3225	0,2167	0,0923	-289,32

When the original collecting dates (converted to JDN) were plotted against year of collection, *N. bulbocodium* and *R. bulbocodium* seem to show a negative relationship (Fig. 3.3), with an advancement of 0,3 and 1,5 days per decade, respectively, but these relationships were not significant. On the contrary, flowering dates of herbarium specimens of *E. australis* seem to be positively correlated with year, with a non-significant relationship indicating a delay of 4,2 days per decade while for *E. arborea* a delay of 6,8 days per decade was observed, with a significant relationship. For all species under consideration, especially in the shrubs, there is a big dispersion of the records.

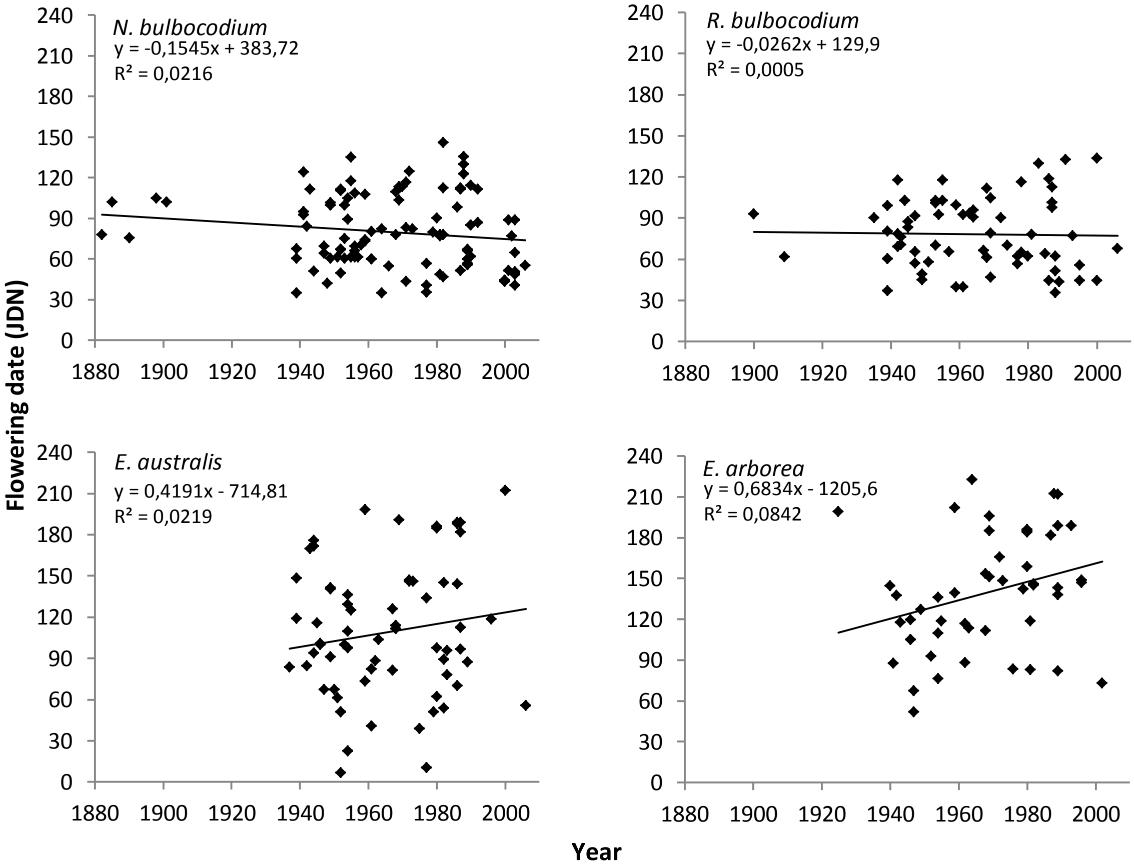

Fig. 3.3 Relationship between the day number that a flowering herbarium specimen of *N. bulbocodium*, *R. bulbocodium*, *E. australis* and *E. arborea* was collected and year.

After applying the corrections to flowering dates using Hopkins's Bioclimatic Law, all the relationships remained statistically not significant, and three of the R^2 even decreased, except one that increased (of *R. bulbocodium*). Furthermore, the general trends of correlation, negative for the bulbous, and positive for the shrub species, remained (Fig. 3.4). Therefore, this analysis indicates an advancement of 0,8 days per decade for *N. bulbocodium*, 10,2 days since 1882 and of 0,5 days per decade for *R. bulbocodium*. For the shrubby species this analysis seems to indicate a delay of 1,4 days per decade for *E. australis* and of 4,2 days per decade for *E. arborea*.

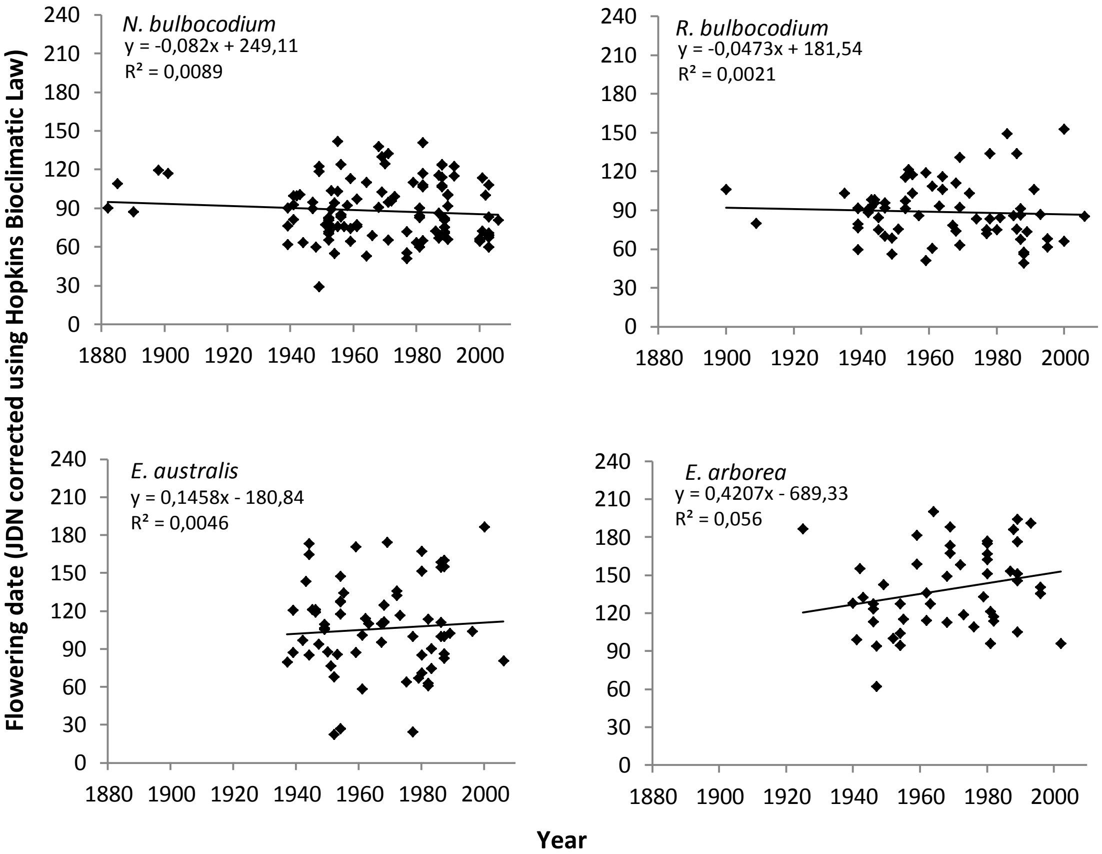

Fig. 3.4 Relationship between the day number that a flowering herbarium specimen of *N. bulbocodium*, *R. bulbocodium*, *E. australis* and *E. arborea* was collected (corrected using Hopkins Bioclimatic Law) and year.

Altitude was found to be a significant predictive term for all the species considered in the multiple regression analysis from the results of the *Stepwise Regression* (Table 3.3). The logarithm of longitude was also found significant for three of the species (*N. bulbocodium*, *E. australis* and *E. arborea*), and latitude was significant only for *E. arborea*. Furthermore, the regression coefficient for altitude (with 95% confidence error bars in Fig. 3.5) showed differences between species according to its habit. The bulbous *N. bulbocodium* and *R. bulbocodium* presented lower values, with 0,0338 and 0,031, respectively, and the shrubs, *E. australis* and *E. arborea* with 0,0541 and 0,0449, respectively.

Table 3.3 Significant predictive terms for all the species considered in the multiple regression analysis. Number of observation (*n*), regression *coefficient*, standard error (*s.e.*), *t* - statistic and *P*-value.

Species		*n*	*coefficient*	*s.e.*	*t*	*P*
Narcissus bulbocodium	constant	117	-67,49	63,91	2	0,293
	Altitude	117	0,038014	0,005045	2	0,000
	log Longitude	117	145,93	68,89	2	0,036
Romulea bulbocodium	constant	70	69,587	3,479	2	0,000
	Altitude	70	0,031222	0,007551	2	0,000
Erica australis	constant	70	360,9	113,3	2	0,002
	Altitude	70	0,05406	0,009226	2	0,000
	log Longitude	70	-321,1	122,3	2	0,011
Erica arborea	constant	51	169,7	218,7	2	0,819
	Altitude	51	0,04488	0,0135	2	0,000
	log Longitude	51	-287,9	149,9	2	0,081
	Latitude	51	5,108	3,43	2	0,103

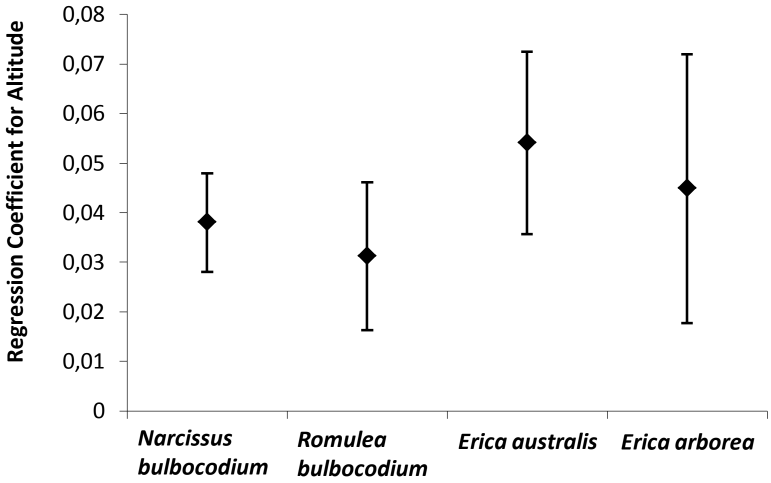

Fig. 3.5 Regression coefficient for Altitude of *N. bulbocodium, R. bulbocodium, E. australis* and *E. arborea*.

The applied corrections to flowering dates using the equations resulting from the Multiple Linear Regressions resulted in the maintenance of the non-significance in two of the relationships (*R. bulbocodium* and *E. australis*) with year, while the same relationship for *E arborea* changed from significant to non-significant and for *N. bulbocodium* changed from non-significant to marginally significant (Fig. 3.6). The general trends of the correlations remained negative for the bulbous species and positive for *E. arborea*, but changed from positive to negative for *E. australis*. Furthermore, this analysis indicates an advancement of 1,4 days per decade for *N. bulbocodium*, of 0,8 days per decade for *R. bulbocodium* and of 0,5 days per decade for *E. australis*. A delay of 2,2 days per decade seems to have happened for *E. arborea*.

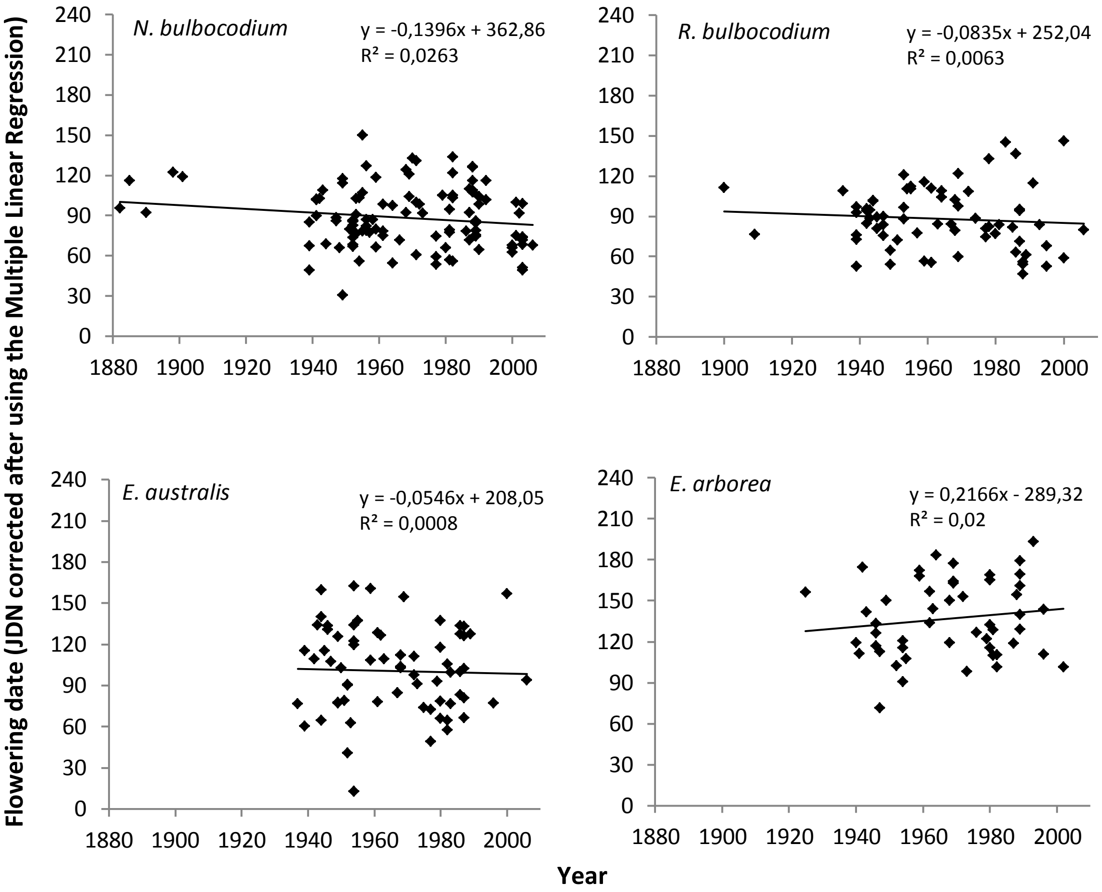

Fig. 3.6 Relationship between the day number that a flowering herbarium specimen of *N. bulbocodium*, *R. bulbocodium*, *E. australis* and *E. arborea* was collected (corrected using the MLR) and year.

The adjustment methods tested, HBL and MLR, were effective in transforming the data collected, dispersed throughout the territory, in data similar to what could have been obtained in one single location, in this case the GCP (Table 3.4). Although the R^2 and P values were progressively worse, the successive approximation of β to 0 when we performed Linear regressions between the original, adjusted using HBL and adjusted using MLR, flowering dates and geographical variables (like altitude in Fig. 3.7), indicates an successive improvement in adjustment of the data across large areas. Regarding the phenologic behavior of the studied species according to the geographic variables, the best fit was observed with altitude using MLR, e.g. (Fig. 3.7).

Table 3.4 Phenologic behavior of the studied species according to the geographic variables (Original data) and adjustment of collection dates (β near to zero indicate the best adjustments) after corrections using HBL and MLR.

Geographic Variables	Original data				Correction using Hopkins's Bioclimatic Law				Correction using the Multiple Linear Regression			
Species	β	α	R^2	P	β	α	R^2	P	β	α	R^2	P
Longitude												
N. bulbocodium	-5,4122	124,26	0,0140	0,2038	4,2498	52,786	0,0127	0,2255	-0,1337	89,289	0,0000	0,9695
R. bulbocodium	-6,2785	131,69	0,0335	0,1294	3,1332	62,163	0,0108	0,3920	1,3346	76,755	0,0019	0,7206
E. australis	-40,489	429,76	0,3346	<0,0001	-22,064	280,63	0,1718	0,0004	0,2515	98,69	0,0000	0,9647
E. arborea	-40,531	466,31	0,4223	<0,0001	-5,3880	182,14	0,0131	0,4238	-0,0158	137,07	0,0000	0,9978
Latitude												
N. bulbocodium	5,9818	-157,52	0,0906	0,0265	-1,6636	153,79	0,0103	0,2752	1,6752	21,777	0,0106	0,2686
R. bulbocodium	4,5506	-101,67	0,0713	0,0255	-1,0832	131,61	0,0052	0,5520	2,8919	-26,396	0,0360	0,1156
E. australis	13,603	-433,65	0,1922	<0,0001	4,6238	-78,589	0,0384	0,1041	0,2803	89,5	0,0002	0,9114
E. arborea	16,101	-507,14	0,3009	<0,0001	1,7110	69,981	0,0060	0,5900	-0,0039	137,1	0,0000	0,9989
Altitude												
N. bulbocodium	0,0334	68,092	0,3120	<0,0001	-0,0029	88,855	0,0035	0,5244	1E-07	88,186	0,0000	1,0000
R. bulbocodium	0,0312	69,587	0,2009	<0,0001	-0,0043	89,959	0,0050	0,5607	4E-07	88,07	0,0000	1,0000
E. australis	0,0674	63,938	0,5097	<0,0001	0,0294	86,223	0,1672	0,0004	-4E-05	100,06	0,0000	0,9961
E. arborea	0,0716	99,292	0,5048	<0,0001	0,0207	127,03	0,0744	0,0529	-1E-05	136,95	0,0000	0,9989

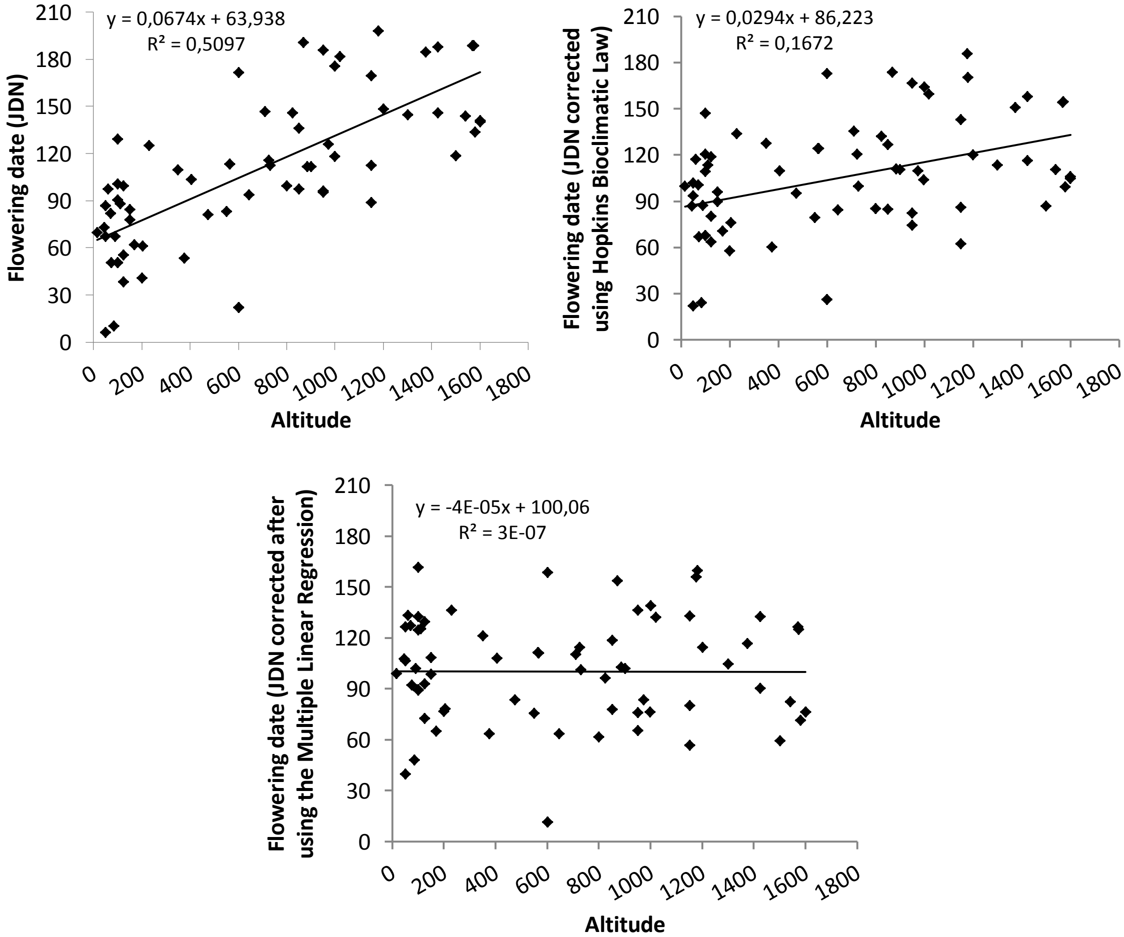

Fig. 3.7 Linear regression models of flowering of *E. australis* with altitude of collection.

Since the XIXth century, the climate in Portugal has become gradually warmer (Fig. 3.8). The mean annual temperature recorded at the Meteorological stations of the Geophysical Institute of Lisbon: Meteorological Observatory of the Serra do Pilar (Geophysical Institute), Meteorological Observatory of the Penhas Douradas, Geophysical Institute of the University of Coimbra and Faro Weather Station / Airport; from 1880 to 2006, rose by approximately 1,8 degrees Celsius in 126 years (y = 0,014x +11,874; R^2 = 0,5418; P <0,0001).

For the same interval of time, 126 years, there was an increase of 1,4 °C in the February to May mean temperature (y = 0,0108x + 9,8912; R^2 = 0,2121; P <0,0001), of 1,4 °C in the January to April mean temperature (y = 0,0115x + 8,2223; R^2 = 0,2689; P <0,0001) and of 1,6 °C in the December to March mean temperature (y = 0,0129x + 7,2728; R^2 = 0,3446; P <0,0001).

Along the years, a great climatic variability was observed (Fig 3.8). In the mean annual temperature (series mean 12,73 °C), the higher peak, reaching 14,25 °C, was observed in 1995, while the lowest peak, with 10,99 °C, was observed in 1889. In the February to May mean temperature (series mean 10,56 °C), the higher peak was observed in 1961, reaching 12,89 °C, and the lower peak was observed in 1890 with a temperature of 8,51 °C. In the January to April mean temperature (series mean 8,95 °C), the higher peak, reaching 10,97 °C, was observed in 1997, while the lower peak, with 6,88 °C, was observed in 1888. Finally, in the December to March mean temperature (series mean 8,08 °C), in 1998, a higher peak was observed, reaching 10,22 °C, and a lower peak, reaching 12,89 °C, was observed in 1888.

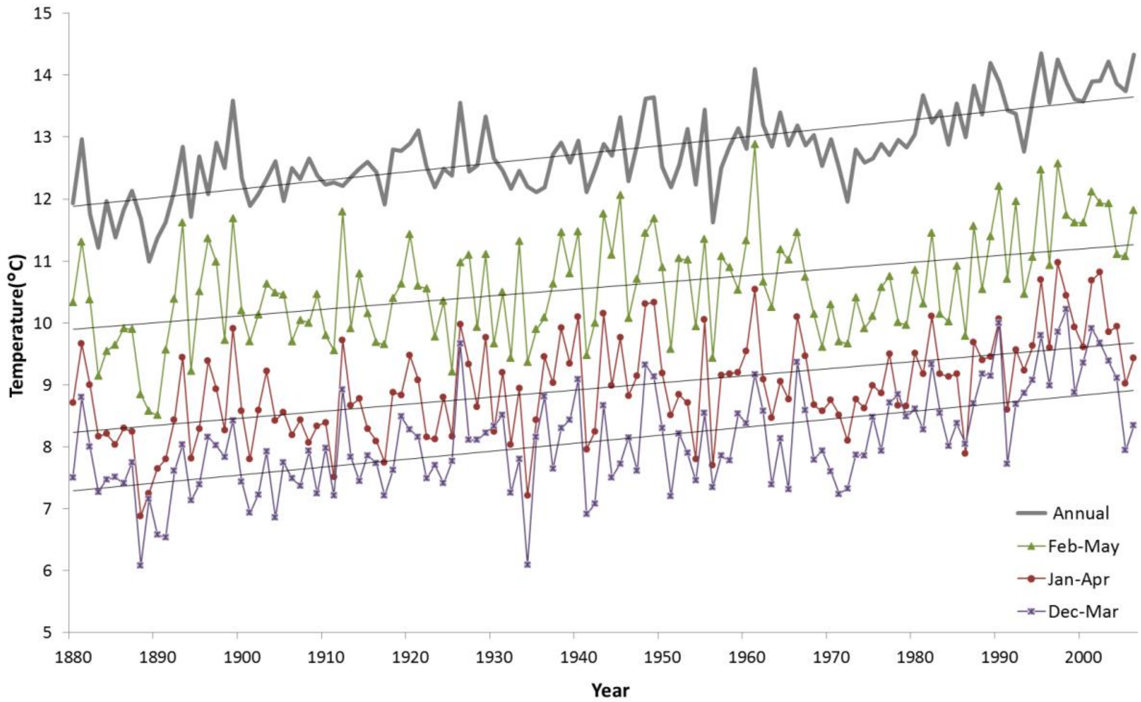

Fig. 3.8 Average temperatures from 1880 to 2006 as reported by Meteorological station of the Geophysical Institute of Lisbon, Meteorological Observatory of the Serra do Pilar (Geophysical Institute), Meteorological Observatory of the Penhas Douradas, Geophysical Institute of the University of Coimbra and Faro Weather Station / Airport. The top series represents mean annual temperatures. The green series represents mean temperature February to May, the red series represents mean temperature January to April, and violet series represents mean temperature December to March. The lines are the best fit lines for the series.

Whereas the relationships between flowering dates and time where mostly non-significant, most of the relationships between flowering dates and mean temperatures where significant (Table 3.5).

Table 3.5 Least-squares linear regression models of flowering observation with temperature of collection. Number of observation (*n*), F-test statistic, R^2 goodness of fit, *P*-value and disturbance term (α). Direction of the trend is indicated by β value where negative numbers represent earlier flowering. Standard error of β is also provided as a measure of confidence in estimates of slope. Type of analysis: Original Data, (Adj. MLR) represents correlation between flowering date (JDN corrected using the MLR) and mean temperature corrected according to the altitude of collecting site and (Adj. after MLR) represents correlation between flowering date (JDN corrected after using the MLR and as if they were measured at the GCP) and mean temperature (corrected as if they were measured at the GCP).

Type of analysis Species	*n*	R^2	*F*	*P*	β	s.e. of β	α
Original data							
N. bulbocodium	117	0,3272	55,9356	<0,0001	-4,9833	0,6663	129,54
R. bulbocodium	70	0,2484	22,4784	<0,0001	-5,0489	1,0649	129,96
E. australis	70	0,5049	69,3439	<0,0001	-10,3910	1,2478	197,45
E. arborea	51	0,5036	49,7186	<0,0001	-10,983	1,5576	257,55
Adj. MLR							
N. bulbocodium	117	0,8784	830,7034	<0,0001	-4,7013	0,1631	126,71
R. bulbocodium	70	0,9307	912,9326	<0,0001	-4,3800	0,1450	123,14
E. australis	70	0,8964	588,6353	<0,0001	-10,324	0,4255	197,08
E. arborea	51	0,8369	251,3762	<0,0001	-10,753	0,6782	255,13
Adj. after MLR							
N. bulbocodium	117	0,0219	2,5707	0,1116	-3,9329	2,4529	121,12
R. bulbocodium	70	0,0783	5,7750	0,0190	-9,5958	3,9930	167,13
E. australis	70	0,0011	0,0741	0,7862	-1,4196	5,2150	112,93
E. arborea	51	0,0089	0,4412	0,5096	-3,8989	5,8697	177,85

The relationships between original flowering dates (converted to JDN) and mean temperature, corrected according to the altitude of collecting site (-6,5 ºC / 1000 m), of the 4 month period selected for each species (for *N. bulbocodium* and *R. bulbocodium* the December-March mean was used, for *E. australis* January-April and for *E. arborea*

February-May) were found to be significant (Fig. 3.9) and all negative, in the period from 1882 to 2006.

For *N. bulbocodium* an advancement of 5 days per 1 °C was observed, for *R. bulbocodium* an advancement of 5 days per 1 °C was observed, for *E. australis* an advancement of 10,4 days per 1 °C and for *E. arborea* an advancement of 11 days per 1 °C was observed.

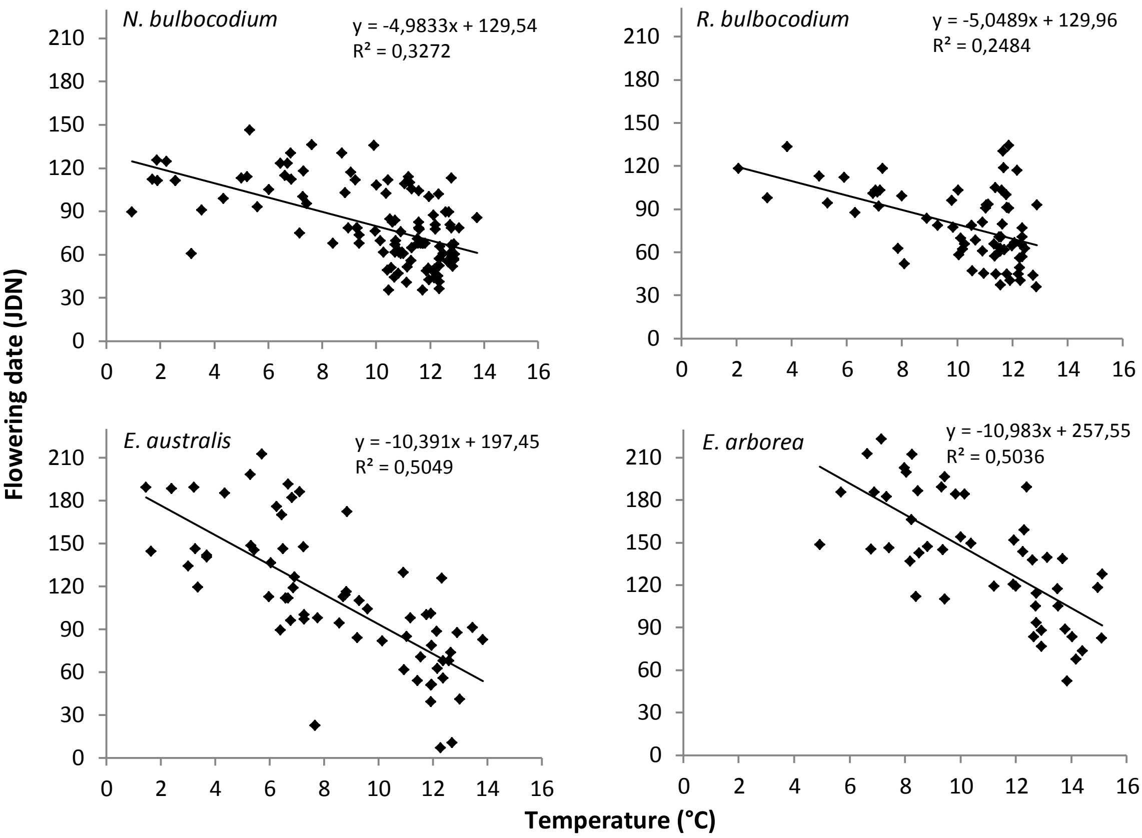

Fig. 3.9 Relationship between flowering date of herbarium specimens (JDN) and mean temperature corrected according to the altitude of collecting site (-6,5 ºC / 1000 m). For *N. bulbocodium* and *R. bulbocodium* the December-March mean was used, for *E. australis* January-April and for *E. arborea* February-May.

Finally, the relationship between flowering date (JDN) of herbarium specimens adjusted using the MLR, and as if they were measured at the GCP, and mean temperature, also adjusted as if measured at the GCP (Fig. 3.10), was non-significant for *N. bulbocodium*, *E.australis* and *E. arborea*, but significant for *R. bulbocodium* (at α level of $P < 0{,}05$).

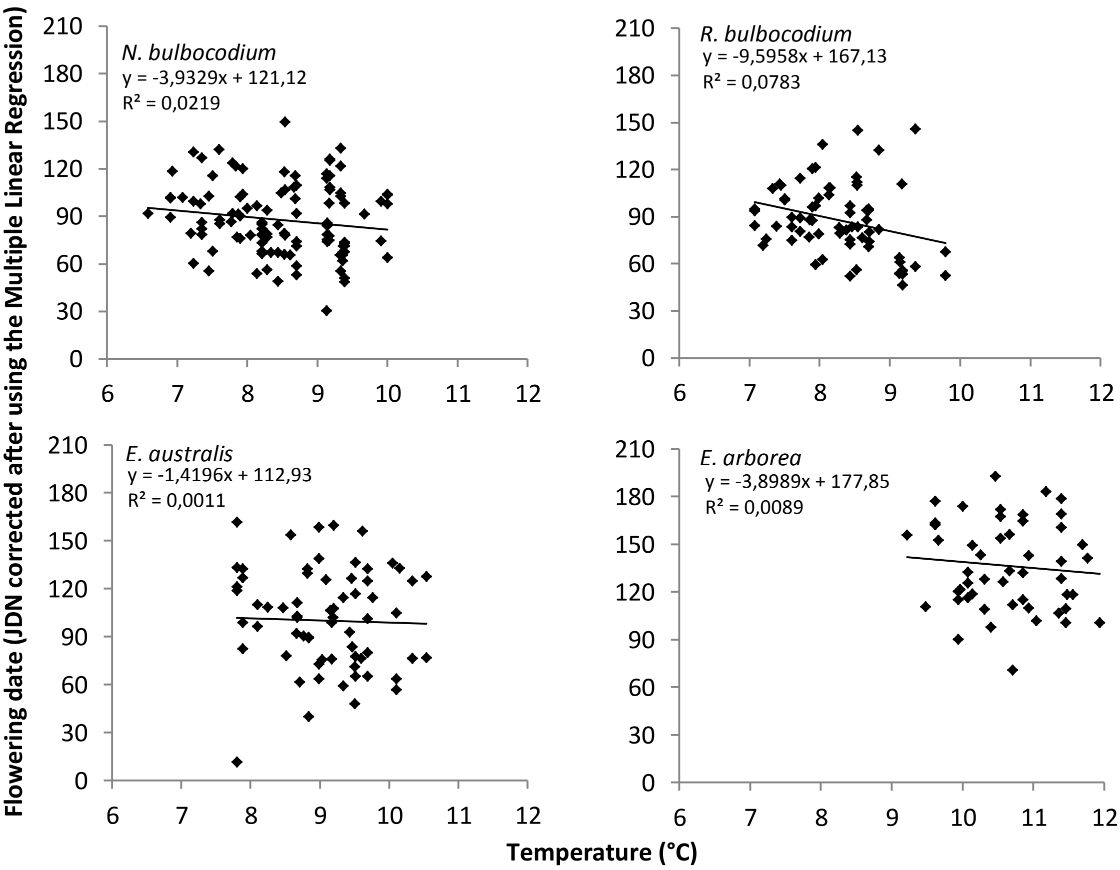

Fig. 3.10 Relationship between flowering date of herbarium specimens (JDN corrected after using the MLR and as if they were measured at the GCP) and mean temperature (corrected as if they were measured at the GCP). For *N. bulbocodium* and *R. bulbocodium* the December-March mean was used, for *E. australis* January-April and for *E. arborea* February-May.

Discussion

Sequentially comparing the graphs over time of the original data with the HBL and with MLRs there is a slight decrease in point dispersion (Fig. 3.3, 3.4, 3.6), however, values of R^2 and P got worse (Table 3.2). To better ascertain the goodness of these adjustments we plotted the new JDN obtained after adjustments in accordance with the HBL or with MLR against the variables altitude, latitude and longitude, and we found that the slope (β) approached zero after these adjustments (Table 3.4), particularly with MLR. This indicates that these corrections improved the adjustment of the data, although microclimatic variability or interference from other variables not considered prevented us from obtaining good fits in the correlations between JDN and year.

Although the relationship between flowering date and year is not significant for all species in the three sets of analysis (Fig. 3.3, 3.4, 3.6), we still considered useful to compare the indicated trends between the different kinds of analysis and with the ones reported in the literature. The most important results from theses analysis is that the deviations of the flowering times across time shifted from advances of 1,5 or 0,3 days per decade for the bulbous species and delays of 4,2 and 6,8 days per decade obtained by plotting the original flowering dates against year to advances of 0,5, 0,8 and 1,4 days per decade, obtained for *E. australis*, *R. bulbocodium* and *N. bulbocodium* (respectively), and a delay of 2,2 days per decade for *E. arborea*, obtained using the MLR to adjust collecting dates. These results seem to agree more with the trend and range reported in the study by Khanduri Sharma & Singh (2008). This author, reviewing the published literature, refers an average advancement of 1,9 days per decade, ranging from 0,2 to 4,5 days per decade, depending on the author, the species and the geographic and temporal scope of the study under review (Khanduri *et al*., 2008).

In Portugal the annual average temperatures and average temperatures of Feb-Mar, Jan-Apr and Dec-Mar increased by 1,8 °C in 126 years, i.e., 0,14 °C per decade. This value agrees with what was reported by Intergovernmental Panel on Climate Change (2007) for the global temperature increase (0,13 °C per decade from 1956 to 2005) and the average temperature increase for Portugal in the period 1941-2005: 0,11 °C per decade (Marques & Antunes, 2009).

To see if this rise in temperature caused an advance in flowering dates of the species under study we first plotted the original flowering dates against the mean temperature corrected to the altitude of collecting site (-6,5 ºC / 100m), and then the adjusted using MLR and temperature as if each specimen had been observed in the GCP. These results reflected the behavior of species facing climate change (Fig. 3.10). However, these correlations were not significant for three of the species. Only for *R. bulbucodium* a significant (*P* = 0,019) relationship was obtained, allowing the use of this species as an indicator for climate change effects on plant phenology. Furthermore, the advance of 9,6 days per 1 ºC temperature increase observed for this species is inside the range of values reported in other studies. For example, Robbirt *et al*. (2011) reported an advance flowering date from 5,7 to 6,7 days per 1 °C increase in temperature, to *Ophris sphegodes*; Gallagher *et al*. (2009) reported an advance range from 4,35 to 11,97 days per 1 °C on Australian alpine species and Primack *et al*. (2004) reported an advance average of 3,9 days per 1 °C in a study with 229 species in Boston. Furthermore, in studies like this, it is usually necessary to screen several species in order to find such an indicator of the effect of climate change on plant phenology. For example, Gallagher, Hughes & Leishman (2009) found a single indicator out of a set of 20 tested among Australian alpine species.

In the study conducted by Panchen *et al*. (2012), species with the shortest flowering season had the greatest amount of their flowering time variation explained by temperature, indicating that species with the shortest flowering times are better indicators of climate change. The flowering times of woody plants were more correlated to temperature than flowering times of herbaceous plants, indicating that woody plants are better indicators of climate change. However, in our study the bulbous obtained better correlation than woody plants, maybe because the *Erica* spp. present a longer flowering period compared with the bulbous species, which confirms that the species with the lowest time of flowering are better indicators of climate change (Miller-Rushing & Primack, 2008).

Some limitations of this study can, however, be identified. The collections of the Portuguese herbaria have a large gap before mid-twentieth century, and a decline in the number of herbarium specimens collected in the decade of 1990s and 2000s, which are among the warmest decades of the last 100 year, (Lavoie & Lachance, 2006, Diskin *et al*., 2012, Panchen *et al.*, 2012). Limited space in herbaria has meant that there has been a hesitancy to collect a large number of specimens, in addition Rumpff *et al.* (2008), also attributed in part to restrictions on collecting imposed by environmental policies. In Portugal, a reduction of funding and shifts in research priorities and policies might also explain the reductions in number of specimens available in some decades, particularly in the recent ones. Microclimate differences across the study area and deviation from the actual peak flowering date are all possible sources of variability (Panchen *et al.*, 2012). As previously mentioned, an additional limitation refers to the use of species with long flowering periods, given the uncertainty in the determination of the peak flowering date (Diskin *et al.,* 2012). Despite *E. australis* and *E. arborea* having a long flowering period and its samples are relatively small, we were able to obtain statistically significant results in response to adjusted mean temperature on collecting site. However, we failed to obtain such a statistically significant relationship between adjusted flowering times after MLR and mean temperature adjusted as if all specimens were collected on the GCP. This was only achieved with the species with the shortest flowering period: *R. bulbocodium.*

It is important to consider the implications of climate change for the higher trophic levels that depend on plant species affected by temperature shifts. Climate warming can negatively affect inter-dependent species such as plants and their insect pollinators due to phenological mismatches (Hegland *et al.*, 2009). Of the screened species, *E. australis* might to be the most relevant to consider, since it is relevant for the production of honey.

Collecting herbarium specimens was very popular around the turn of the twentieth century, but taking digital photographs is now very popular (Panchen *et al*., 2012). However, the enrichment of herbarium collections should continue to avoid any gap in biological records thus compromising its quality (Lavoie & Lachance, 2006, GairaDhar & Belwal, 2011). Herbaria worldwide contain millions of specimens, the potential for future studies is enormous, representing an as yet underexplored resource. Digitalization would increase the accessibility of herbarium specimens, facilitating remote research efforts and could thus prove to be a cost-effective way of increasing sample size (Diskin *et al*., 2012). If these collections can be associated to an efficient method to adjust differences in collecting sites, such as the one presented here, the power becomes even greater.

In conclusion, the data adjustment of herbarium specimens collected in a large area was obtained with a high degree of significance using the MLR Model. The use of herbarium specimens is recommended as a robust methodology, if care is taken to select a species with a short flowering time and a large sample size from which significant results can be elucidated.

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